Role of Melatonin and Lighting Conditions in Improving the Growth Traits of *Gardenia jasminoides* Ellis Cultured *in Vitro*

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Abstract

This study was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture and Landscape Gardening, College of Agriculture, Al-Qasim Green University, Iraq, from September 2025 to June 2026 to evaluate the effects of lighting conditions and melatonin concentrations on the growth characteristics of *Gardenia jasminoides* shoots cultured *in vitro*. The experiment consisted of two factors with ten replicates: three lighting conditions (white fluorescent, blue LED, and combined red–blue LED) and four melatonin concentrations (0, 0.6, 0.8, and 1.0 mg L^{-1}). Melatonin significantly improved most of the studied growth characteristics; 1.0 mg L^{-1} produced the highest shoot length, shoot number, and leaf number, whereas 0.8 mg L^{-1} resulted in the highest dry matter percentage and 0.6 mg L^{-1} gave the highest chlorophyll content. Lighting conditions also significantly affected plant growth, with the combined red–blue LED treatment producing the highest shoot length, shoot number, leaf number, and dry matter percentage, while red LED light alone resulted in the highest chlorophyll content. Significant interaction effects were observed between melatonin and lighting conditions for all growth characteristics. The combination of 1.0 mg L^{-1} melatonin with red–blue LED light produced the highest shoot length, shoot number, and leaf number, whereas 0.8 mg L^{-1} melatonin combined with red–blue LED light resulted in the highest dry matter percentage, and 0.6 mg L^{-1} melatonin combined with red LED light produced the highest chlorophyll content compared with the control.

Keywords: Plant Tissue Culture, *Gardenia jasminoides*, Melatonin, LED Lighting

1- Introduction

Gardenia is an economically and medicinally important ornamental shrub belonging to the family Rubiaceae. Native to East Asia, particularly China, it is highly valued for its fragrant white flowers and glossy green foliage, making it one of the most widely cultivated ornamental plants for landscape design, perfume production, and cosmetic industries. In addition, increasing attention has been directed toward this species because of its rich content of biologically active compounds with recognized medicinal value [1];[2].

Plant tissue culture is considered one of the most efficient techniques for plant propagation because it provides controlled growth conditions and a uniform environment that minimizes the influence of external environmental factors. However, the success of in vitro culture largely depends on optimizing factors affecting tissue growth, particularly

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lighting conditions and plant growth regulators, owing to their fundamental roles in regulating plant growth and development. Light is a key environmental factor controlling the growth of in vitro-cultured plants through its direct influence on photosynthesis, cell division, and numerous physiological and biochemical processes. Among the different wavelengths, red and blue light are regarded as the most effective for promoting plant growth and micropropagation due to their essential roles in regulating photochemical reactions and plant development[3]. Although conventional tissue culture systems mainly rely on broad-spectrum fluorescent lamps (350–750 nm), these lighting systems are limited by lower spectral efficiency, higher energy consumption, and excessive heat production. Consequently, light-emitting diode (LED) technology has become increasingly adopted because of its high energy efficiency and precise control of light spectra, providing more suitable lighting conditions for improving plant growth under in vitro conditions [4].

Melatonin has recently emerged as an important plant growth regulator in tissue culture because of its ability to enhance the growth and development of cultured tissues through the regulation of hormonal balance and interactions with other endogenous phytohormones, thereby improving propagation efficiency and plant growth. Moreover, melatonin enhances photosynthetic efficiency and increases plant tolerance to in vitro conditions, ultimately improving plant vigor and growth quality[5],[6]. Therefore, the present study aimed to investigate the effects of different lighting conditions and melatonin on the growth characteristics of Gardenia cultured *in vitro* in order to identify the most effective treatment for improving micropropagation efficiency and plant growth under laboratory conditions.

2-Materials and Methods

The experiment was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture and Landscape, College of Agriculture, Al-Qasim Green University, from September 2025 to June 2026 to investigate the effects of lighting conditions and melatonin on selected growth characteristics of Gardenia cultured *in vitro*. Soft shoots (5–10 cm long) were collected from healthy gardenia plants and transferred to the tissue culture laboratory. The shoots were defoliated, cut into 2-cm segments, and rinsed under running tap water for 30 min to remove dust and surface contaminants. Surface sterilization was carried out in a laminar airflow cabinet by immersing the explants in 70% ethanol for 10 s, followed by 1% sodium hypochlorite (NaOCl) supplemented with two drops of Tween-20 for 15 min with continuous agitation. The explants were then rinsed three times with sterile distilled water to eliminate residual disinfectant.

Following surface sterilization, the explants were trimmed to approximately 1 cm, each containing a single node, and cultured vertically in glass culture vessels containing Murashige and Skoog (MS) medium supplemented with 30 g L^{-1} sucrose, 100 mg L^{-1} myo-inositol, and 7 g L^{-1} agar. Cultures were maintained at 25 ± 2°C under a 16-h photoperiod, an 8-h dark period, and a light intensity of 1000 lux. After shoot establishment, single nodal segments were subcultured onto the same MS medium supplemented with 2 mg L^{-1} 6-benzyladenine (BA) and 0.1 mg L^{-1} α-naphthaleneacetic acid (NAA). Subculturing was repeated under the same environmental conditions until sufficient

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plant material was obtained for the experiment.

The experiment was arranged using four melatonin concentrations (0, 0.6, 0.8, and 1.0 mg L^{-1}) and three lighting conditions: white fluorescent lamps, red LED lamps, and combined red–blue LED lamps at a 4:1 ratio. The culture medium consisted of MS salts supplemented with 30 g L^{-1} sucrose, 100 mg L^{-1} myo-inositol, 2 mg L^{-1} BA, 0.1 mg L^{-1} NAA, and 7 g L^{-1} agar, in addition to the respective melatonin treatments. Following medium sterilization, explants were cultured in glass vessels containing 10 mL of culture medium, with ten replicates per treatment. Cultures were incubated at 25 ± 2°C under the designated lighting conditions with a 16-h light/8-h dark photoperiod for six weeks. At the end of the incubation period, shoot length, shoot number, leaf number, chlorophyll content, and dry matter percentage were recorded.

3.Results

3-1 Shoot Length (cm plantlet^{-1})

The results presented in Table 1 showed that melatonin significantly affected shoot length. The highest mean shoot length (6.86 cm) was obtained with 1.0 mg L^{-1} melatonin, whereas the control treatment produced the lowest mean value (6.00 cm). Lighting conditions also had a significant effect on this trait, with the combined red–blue LED treatment producing the greatest mean shoot length (6.87 cm), while white fluorescent light resulted in the lowest mean value (6.06 cm). A significant interaction was also observed between melatonin and lighting conditions. The combination of 1.0 mg L^{-1} melatonin and red–blue LED light produced the highest mean shoot length (7.50 cm), whereas the control treatment under white fluorescent light recorded the lowest mean value (5.50 cm).

Table 1. Effects of melatonin, lighting conditions, and their interaction on shoot length of Gardenia cultured *in vitro* (cm).

Melatonin (mg L^{-1})	Light Source			Mean of Melatonin
	White Fluorescent	Red LED	Red + Blue LED	
0.0	5.50	6.16	6.34	**6.00**
0.6	6.00	6.33	7.00	**6.44**
0.8	6.50	6.84	6.66	**6.67**
1.0	6.25	6.83	7.50	**6.86**
Mean of Light	**6.06**	**6.54**	**6.87**	
0.5301 = **Light Source** 0.4591 = **Melatonin** 0.9182 = **Interaction**				**LSD (0.05)**

3-2 Number of Shoots (shoots plantlet^{-1})

The results presented in Table2 revealed significant differences among melatonin concentrations in shoot number. The highest mean (7.33 shoots plant^{-1}) was recorded at 1.0 mg L^{-1} melatonin, whereas the control treatment produced the lowest mean (4.00 shoots plant^{-1}). Lighting conditions also significantly

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The combination of 1.0 mg L^{-1} melatonin and red–blue LED light produced the highest mean (9.50 shoots $plant^{-1}$), whereas the control treatment under white fluorescent light recorded the lowest mean (2.00 shoots $plant^{-1}$).

affected shoot number, with the combined red–blue LED treatment producing the highest mean (8.00 shoots $plant^{-1}$), while white fluorescent light resulted in the lowest mean (4.75 shoots $plant^{-1}$). A significant interaction was observed between melatonin and lighting conditions.

Table 2. Effects of melatonin, lighting conditions, and their interaction on number of shoots of Gardenia cultured *in vitro* (shoots $plantlet^{-1}$).

Melatonin (mg L^{-1})	Light Source			Mean of Melatonin
	White Fluorescent	Red LED	Red + Blue LED	
0.0	2.00	5.00	5.00	**4.00**
0.6	5.00	7.5	9.00	**7.17**
0.8	6.50	7.00	8.50	**7.33**
1.0	5.50	7.00	9.50	**7.33**
Mean of Light	**4.75**	**6.62**	**8.00**	
0.702 = **Light Source** 0.608 = **Melatonin** 1.216 = **Interaction**				**LSD (0.05)**

3.3 Number of Leaves (leaves $plantlet^{-1}$)

$plant^{-1}$), while white fluorescent light resulted in the lowest mean (22.38 leaves $plant^{-1}$). A significant interaction was observed between melatonin and lighting conditions. The combination of 1.0 mg L^{-1} melatonin and red–blue LED light produced the highest mean (53.00 leaves $plant^{-1}$), whereas the control treatment under white fluorescent light recorded the lowest mean (19.00 leaves $plant^{-1}$)

The results presented in Table 3 showed that melatonin significantly affected leaf number. The highest mean (40.83 leaves $plant^{-1}$) was obtained with 1.0 mg L^{-1} melatonin, whereas the control treatment recorded the lowest mean (24.00 leaves $plant^{-1}$). Lighting conditions also exerted a significant effect on leaf number. The combined red–blue LED treatment produced the highest mean (41.00 leaves

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Table 3. Effects of melatonin, lighting conditions, and their interaction on number of leaves of Gardenia cultured *in vitro* (leaves plantlet^{-1}).

Melatonin (mg L^{-1})	light source			Mean of Melatonin
	White Fluorescent	Red LED	Red + Blue LED	
0.0	**19.00**	**22.50**	**30.50**	**24.00**
0.6	**21.50**	**43.50**	**33.50**	**32.83**
0.8	**22.50**	**24.00**	**47.00**	**31.17**
1.0	**26.50**	**43.00**	**53.00**	**40.83**
Mean of Light	**22.38**	**33.25**	**41.00**	
2.189 = **light source** 1.896 = **Melatonin** 3.792 = **Interaction**				**LSD (0.05)**

4.3 Chlorophyll Content (mg 100 g^{-1} FW)

fluorescent light resulted in the lowest mean value (40.072 mg g^{-1} FW). A significant interaction was observed between melatonin and lighting conditions. The combination of 0.6 mg L^{-1} melatonin and red LED light produced the highest mean chlorophyll content (48.648 mg g^{-1} FW), whereas the control treatment under white fluorescent light recorded the lowest mean value (37.202 mg g^{-1} FW).

The results presented in Table 4 showed that melatonin significantly affected chlorophyll content. The highest mean chlorophyll content (44.547 mg g^{-1} FW) was recorded at 0.6 mg L^{-1} melatonin, whereas the control treatment produced the lowest mean value (39.847 mg g^{-1} FW). Lighting conditions also had a significant effect on chlorophyll content, with red LED light producing the highest mean (44.146 mg g^{-1} FW), while white

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able 4. Effects of melatonin, lighting conditions, and their interaction on total chlorophyll content of Gardenia cultured *in vitro* (mg 100 g^{-1} FW).

Melatonin (mg L^{-1})	light source			Mean of Melatonin
	White Fluorescent	Red LED	Red + Blue LED	
0.0	37.202	41.355	40.986	**39.847**
0.6	40.857	48.648	44.134	**44.547**
0.8	40.267	44.573	44.220	**43.020**
1.0	41.962	42.006	44.275	**42.748**
Mean of Light	**40.072**	**44.146**	**43.404**	
0.5194 = **light source** 0.4498 = **Melatonin** 0.8996 = **Interaction**				**Least LSD (0.05)**

5.3 Dry Matter Percentage (%)

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(16.30%), while white fluorescent light resulted in the lowest mean (14.73%). A significant interaction was also detected between melatonin and lighting conditions. The combination of 0.8 mg L^{-1} melatonin and red–blue LED light produced the highest dry matter percentage (17.84%), whereas the control treatment under white fluorescent light recorded the lowest mean (12.51%).

The results presented in Table 5 showed that melatonin significantly affected dry matter percentage. The highest mean value (16.70%) was obtained with 0.8 mg L^{-1} melatonin, whereas the control treatment recorded the lowest mean (13.37%). Lighting conditions also exerted a significant effect on dry matter percentage. The combined red–blue LED treatment produced the highest mean

Table 5. Effects of melatonin, lighting conditions, and their interaction on dry matter percentage of Gardenia cultured *in vitro* (%).

Melatonin (mg L^{-1})	light source			Mean of Melatonin
	White Fluorescent	Red LED	Red + Blue LED	
0.0	12.51	14.13	13.47	**13.37**
0.6	14.70	16.43	17.65	**16.26**
0.8	16.29	15.96	17.84	**16.70**
1.0	15.41	15.99	16.26	**15.89**
Mean of Light	**14.73**	**15.63**	**16.30**	
1.039 = **light source** 0.900 = **Melatonin** 1.800 = **Interaction**				**LSD (0.05)**

4.Discussion

accumulation and increases dry matter production[7];[8];[9]. Furthermore, melatonin enhances chlorophyll biosynthesis and preserves the efficiency of photosystem II (PSII), thereby sustaining photosynthetic activity and promoting vegetative growth[10]. These findings are consistent with those reported by [11]and [12].

The results further showed that the combined red–blue LED treatment significantly enhanced vegetative growth compared with white fluorescent light, resulting in greater shoot length, shoot number, leaf number, chlorophyll content, and dry matter percentage. This response is likely associated with the complementary roles of red and blue wavelengths in regulating physiological

The results of the present study demonstrated that melatonin significantly improved all the evaluated growth characteristics, including shoot length, shoot number, leaf number, chlorophyll content, and dry matter percentage, compared with the control treatment. These improvements may be attributed to the role of melatonin in promoting cell division and cell elongation through its interaction with indole-3-acetic acid (IAA), in addition to its ability to regulate the expression of genes involved in auxin transport and signaling, thereby enhancing meristematic activity and the development of different plant organs. Melatonin also contributes to improving photosynthetic efficiency by maintaining chloroplast integrity and enhancing the antioxidant defense system, which promotes biomass

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meristematic activity and stimulates lateral bud development, leading to improved vegetative growth. These findings are in agreement with those reported by [13]in banana, [14]in citrus, and [15]in date palm.

5.Conclusion

produced greater improvements in growth than either factor applied individually, emphasizing the importance of integrating these treatments to enhance the efficiency of micropropagation and improve the quality of plantlets produced under *in vitro* conditions.

processes related to plant growth, stimulating cell division and elongation, improving photosynthetic efficiency, increasing carbohydrate production, and enhancing dry matter accumulation. In addition, this lighting regime promotes

The results of this study demonstrated that both melatonin and lighting conditions play essential roles in regulating the growth of *Gardenia* shoots cultured *in vitro*. Melatonin significantly improved the evaluated growth characteristics, while red LED and combined red–blue LED lighting promoted vegetative growth and increased chlorophyll content compared with white fluorescent light. Moreover, the interaction between melatonin and lighting conditions



Figure (2) Sterilization of plant medium (MS medium).

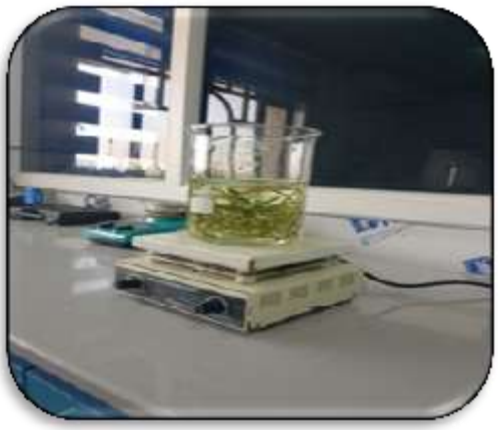


Figure (1). Preparation of the culture explants prior to tissue culture.

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Figure (3) Culturing of plant explants on the culture medium.



Figure (4). Multiplication of plant explants under in vitro conditions.

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Figure (5). Incubation of plant explants under different light sources.

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6.References

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